

RESEARCH ARTICLE

Development of a Topically Active Imiquimod Formulation

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ABSTRACT

The purpose of this work was to develop a topical formulation of imiquimod, a novel immune response modifier, to induce local cytokine production for the treatment of external genital and perianal warts. A pH-solubility profile and titration data were used to calculate a pK_a of 7.3, indicative of a weak base. Solubility experiments were conducted to identify a solvent that dissolves imiquimod to achieve a 5% formulation concentration. Studies to select surfactants, preservatives, and viscosity-enhancing excipients to formulate an oil-in-water cream indicated that fatty acids were the preferred solvent for topical imiquimod formulations, and isostearic acid (ISA) was selected. A relationship existed between the fatty acid composition of four commercially available ISA sources and the solubility of imiquimod. A combination of polysorbate 60, sorbitan monostearate, and xanthan gum was used to produce a physically stable cream. The preservative system included parabens and benzyl alcohol to meet the USP criteria for preservative activity. An *in vitro* method was developed to demonstrate that imiquimod was released from the formulation. Topical application of the formulation induced local cytokine activity in mice.

KEY WORDS: Cytokines; Emulsion; Imiquimod; Isostearic acid; Solubility.

INTRODUCTION

Genital warts, a sexually transmitted disease caused by human papilloma virus (HPV), affects millions of people and the number of new cases grows significantly each year. The condition may present psychological barriers

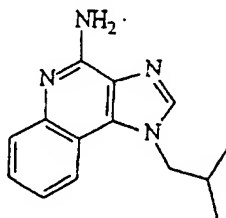
that can prevent patients from seeking treatment. The traditional treatment options for this growing health problem are limited, painful, and tissue-destructive (surgical or caustic agents). A new class of drugs has been discovered which activates the immune system to clear these warts. Imiquimod (R-837 or S-26308, 3M Pharmaceuti-

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cals) has been shown in animals and human cell lines to induce cytokine production from monocytes and macrophages, including interferon- α (IFN- α), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) (1,2). Imiquimod topical cream stimulates local production of IFN- α and TNF- α when applied to the skin of mice and rats. The compound is topically effective in rodents against some viral infections including HPV, cytomegalovirus (CMV), and herpes simplex virus (HSV) (3,4).

A preferred topical product that is applied to the skin containing hair follicles should be water-washable and cosmetically elegant to encourage patient compliance, and should provide the correct flux of drug into the site of action (5). A topical formulation of imiquimod was desired to provide local cytokine induction.

Imiquimod is a small molecule (molecular weight 240.3) that is practically insoluble in water and sparingly soluble in other common pharmaceutical solvents.



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Structure 1. Imiquimod.

The ionization constant of the conjugate acid (pK_a) is 7.3, as determined by titration data and pH solubility curves. It is a chemically stable off-white crystalline powder with a high melting point (297–299°C). Given the poor solubility characteristics of the molecule, it was not possible to solubilize enough drug to form a therapeutically effective single-phase gel. The formulation efforts described in this paper focused on developing an oil-in-water (o/w) emulsion for topical application. The purpose of this work was to develop a topical formulation of imiquimod that when applied topically, induces local cytokine production.

Fatty acids can be used as solubilizing agents for topical formulations; stearic acid and oleic acid are common in dermatological preparations throughout the world. Isostearic acid (ISA), a mixture of branched saturated fatty acids, has a low melting point (10°C) and good stability against oxidation and rancidity. It is a clear oily liquid at room temperature and is immiscible with water. Although not a compendial pharmaceutical excipient, it

is used frequently in topical cosmetic products such as makeup, lipstick, sunscreens, moisturizing lotions, and shaving creams. Numerous toxicity tests have shown that ISA is safe for topical use on human skin (6), and in fact, human skin itself contains many free and esterified fatty acids of a similar chain length to the components found in commercial ISA (7). A U.S. patent was issued in 1993 relating to formulations containing imiquimod and ISA (8).

MATERIALS AND METHODS

Materials

ISA Emersol 875 grade was purchased from Henkel (Cincinnati, OH). ISA Unimate 2000 grade was purchased from Union Camp Corp. (Dover, OH). ISA Pri-sorine 3505 grade was purchased from Unichema (Chicago, IL). ISA chemical grade was purchased from Sigma (St. Louis, MO). Imiquimod was synthesized by 3M Pharmaceuticals (St. Paul, MN). Concentrated hydrochloric acid was purchased from Fisher Scientific (Itasca, IL) and HPLC grade methanol was purchased from EM Sciences (Gibbstown, NJ). All surfactants were purchased from Sigma. Xanthan gum was purchased from Kelco (Chicago, IL). All other materials were laboratory grade and used as received.

Methods

Preparation of Emulsions

The solids were dissolved in either the oil or aqueous phase. Each phase was heated to about 70°C and homogenized with a Silverson model L4R homogenizer with a high-shear screen on the highest setting for about 20 min. After homogenization, the creams were stirred with a paddle and cooled to 25°C.

pH-Solubility Profile

The pH-solubility profile of imiquimod was determined in modified Britton–Robinson buffer over the range of pH 2.20–9.59 (9). Samples were prepared by the addition of approximately 100 mg of imiquimod to 20 ml of buffer in a glass vial. The vials were shaken for 90 hr and the temperature was maintained at $24 \pm 2^\circ\text{C}$. The suspension was filtered, the filtrate was diluted to an appropriate concentration with 0.1 N HCl, and the sample absorbance was measured at 225 nm. A reference solution of 0.1 N HCl was used unless the dilution was less than 1 to 25, in which case a reference solution of the

appropriate buffer solution and 0.1 N HCl was prepared at the same dilution as the sample. The pH of each sample was measured after the sample was removed from the shaker.

Imiquimod Solubility in Organic Acids

The solubility of imiquimod was estimated in organic acids after a suspension of imiquimod in a solvent was shaken for at least 30 min at room temperature. The suspension was filtered through a 0.22- μ m poly(tetrafluoroethylene) (PTFE) filter and the concentration was measured in the filtrate with an HPLC assay.

Analysis of ISA

The composition of ISA was determined with a gas chromatographic procedure. The assay employed a gas chromatograph (GC) (HP5890 Series II, Hewlett-Packard, Avondale, PA) with a 30 m \times 0.53 mm i.d., 0.25- μ m film thickness Stabilwax DA capillary column (Restek, Bellefonte, PA). GC operating conditions were as follows: 120°C initial temperature, 4.0°C/min ramp to 220°C, then isothermal at 220°C for 10 min; injector temperature was 210°C; flame ionization detector temperature was 250°C; and nitrogen carrier gas was at 25 ml/min. Samples were prepared by diluting approximately 300 mg of ISA in 50 ml of methylene chloride.

Choice of Excipients

Surfactant systems were evaluated after the prepared cream was placed in several temperature conditions. The viscosity and appearance of the formulation were monitored as a function of time. The viscosity measurement was a single-point method using a Brookfield viscometer with an LVT head and no. 5 spindle at 30 rpm for 10 min on a sample at room temperature. Several preservative systems were evaluated using the USP 23 criteria for preservative effectiveness testing.

Imiquimod Solubility in ISA

An excess of imiquimod was added to ISA in a glass vial and sealed with a PTFE-lined lid. The vials were placed in a shaking waterbath for at least 48 hr. The equilibrium solubility was measured in the temperature range from 30 to 70°C. After 48 hr, samples were filtered with a 0.45- μ m PTFE filter and an aliquot of the filtrate was placed in a volumetric flask. A sufficient volume of a dilution solution containing 90% methanol, 9% water, and 1% HCl was added to the volumetric flask. These solutions were stirred with a stir bar for at least 10 min for

complete dissolution. The samples were analyzed with a UV spectrophotometer with a 1-cm pathlength cuvette at a wavelength of 320 nm, or by HPLC with UV detection.

pK_a Determination

Methanol-water solutions were prepared at 10, 15, and 20% by volume containing a standard concentration of imiquimod and hydrochloric acid. Exactly 80 g of each solution was titrated with 0.01 N NaOH using a Mettler DL125 titrator. The apparent pK_a in methanol-water solutions was calculated using the Henderson-Hasselbach equation, and the pK_a in water was determined by extrapolating to zero methanol concentration. The pK_a of imiquimod in water was determined at room temperature (~22), 25, 30, 40, 50, and 60°C.

Imiquimod Release Method

Approximately 0.75 g of formulation was placed on 2.0 cm² of a microporous polyethylene membrane within a modified Franz diffusion cell manufactured at 3M. The receptor fluid, 11 ml of 0.1 N hydrochloric acid, was stirred with a PTFE-coated bar magnet. The Franz cells were placed in a chamber controlled at 32 \pm 1°C and 50 \pm 15% relative humidity. The receptor media was removed at specified time intervals and replaced with fresh media. The receptor media was analyzed for imiquimod concentration with a UV assay at 319 nm and a 1-cm cell. The flux was calculated from the total amount diffused per time and corrected for membrane area.

Pharmacological Effectiveness of Topical Cream

A 5% formulation of imiquimod cream (30 μ l) was applied to the left flank of hairless SKH-1 male mice (Charles River, Portage, MI) that were about 6–8 weeks of age. The animals were jacketed to prevent ingestion of the drug. Biopsies of 200 mg were removed from the dosed skin and assayed for IFN- α , TNF- α , and IL-6 according to previously published methods (10). The results were compared to cytokine levels found in mouse skin taken from a site not exposed to drug.

RESULTS AND DISCUSSION

Suspension of Imiquimod in a Gel

The first topical formulation of imiquimod used in pre-clinical pharmacology studies was a gelled aqueous suspension of the drug. Imiquimod was micronized to max-

imize surface area and facilitate delivery. The micronized drug was suspended in water at concentrations of 1, 3, and 5% using colloidal cellulose (5%), and preserved with methylparaben and propylparaben. The resulting gels, when applied to the skin of rodents, proved ineffective at inducing pharmacological activity (cytokine release and antiviral activity). However, solutions of the compound dissolved in ISA when applied to the skin of guinea pigs demonstrated antiviral activity. Subsequent studies using emulsions of ISA in water in which imiquimod was fully dissolved in the oil phase showed skin permeability *in vitro* and ultimately led to the marketed cream formulation. Once it was demonstrated that imiquimod needed to be formulated as a solution or emulsion, to produce activity, formulation efforts focused on evaluation of the potential solubilizing agents for the drug.

Solubility Characteristics of Imiquimod

Preliminary clinical studies demonstrated that a target concentration of 5% was desired for the topical formulation of imiquimod. The aqueous solubility of imiquimod was assessed at several pH conditions and imiquimod was found to be highly insoluble in aqueous systems. In acidic environments, the solubility increased as imiquimod was converted into the corresponding salt form. The solubility of imiquimod in water as a function of pH is shown in Fig. 1. At pH greater than 5, this is a typical profile for a weak base, and the calculated pK_a of imiquimod was 7.3. The plateau effect at pH less than 5 is a result of the formation of less-soluble salts from the

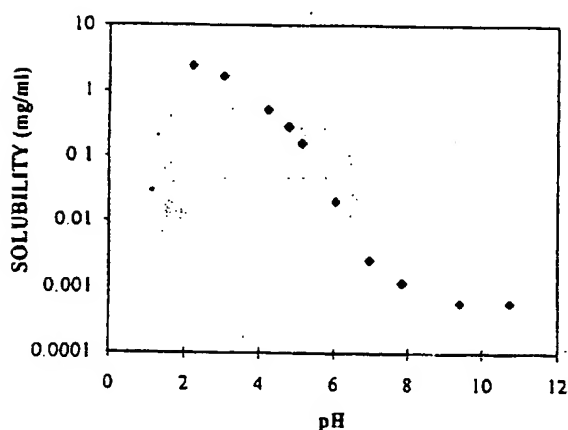


Figure 1. Aqueous solubility of imiquimod as a function of pH using Britton–Robinson buffer.

Table 1
Solubility of Imiquimod in Selected Organic Solvents

Solvent	Solubility (mg/ml)
Isopropyl palmitate	0.01
Acetonitrile	0.055
Sorbitan monooleate	0.06
Acetone	0.12
Propylene glycol	0.12
Ethanol	0.24
PEG 400 monoisostearate	0.33
Methanol	0.46
Chloroform	0.56
Dimethylformamide	0.76
N-Methyl-2-pyrrolidone	1.0
ISA	17
Linoleic acid	17
Oleic acid	20

buffer system. Even at extremely low pH conditions, it was not possible to solubilize imiquimod in an aqueous environment to achieve a 5% concentration. Therefore, water-immiscible solvents were screened for their ability to solubilize imiquimod for an o/w emulsion formulation.

More than 70 organic solubilizers and lipid mixtures were screened to determine the solubility of imiquimod in these solvents. Selected solvents that illustrate the poor solubility of imiquimod are shown in Table 1. The highest solubilities were seen with long-chain fatty acids such as oleic acid, linoleic acid, and ISA. Commercial oleic acid (melting point 4°C) is composed mainly of unsaturated fatty acids, with 7–12% saturated acids allowed in the compendial grades. It is a common emulsifier and solubilizer in pharmaceutical formulations, but oxidizes in air when heated, turning yellow and producing a rancid odor. Commercial linoleic acid (melting point 12°C) is also primarily unsaturated and exhibits similar instability problems in the presence of air, especially upon heating. Most o/w emulsion manufacturing procedures mix the two phases at elevated temperatures, making unsaturated fatty acids less suitable because of the potential for oxidation. Stearic acid USP is a mixture of palmitic and stearic acids that melts at 54°C; pure stearic acid melts at 69°C. Excluding micellar solubilization, this makes stearic acid unsuitable for solubilizing imiquimod in the oil phase at room temperature.

ISA (melting point 10°C) is a mixture of branched (mostly methyl-branched) and straight-chain isomers of

C_{18} , C_{16} , and C_{14} saturated fatty acids of the general formula $C_{17}H_{35}COOH$. ISA is less susceptible to color change and oxidation than the unsaturated fatty acids and has many of the properties of stearic acid. However, it has the fluidity and solubilizing properties of oleic acid. It is immiscible with water, providing a good substrate for the dispersed phase of an o/w emulsion, and it has the ability to solubilize compounds that are not water soluble. The Emersol 875 grade of ISA was chosen as the oil phase and solubilizer for imiquimod cream. It was the most resistant grade to oxidative degradation and had the lightest color and least odor.

Composition of Commercial ISA

The solubility of imiquimod in ISA varied slightly depending upon the source of the ISA. The fatty acid composition of ISA was profiled with a GC procedure. Typical chromatograms are shown in Fig. 2. Table 2 summarizes the variability of fatty acid composition among the specified vendors of ISA. The Emersol 875 grade of ISA contained relatively more short chain fatty acids than the ISAs from the other three vendors. Analysis of multiple batches of Emersol 875 showed this mixture to be consistent from batch to batch. The solubility of imiquimod was determined in each of these lots of ISA, and a correlation was found between imiquimod solubility in ISA and fatty acid composition of the ISA. The solubility of imiquimod was greater in ISA that contained

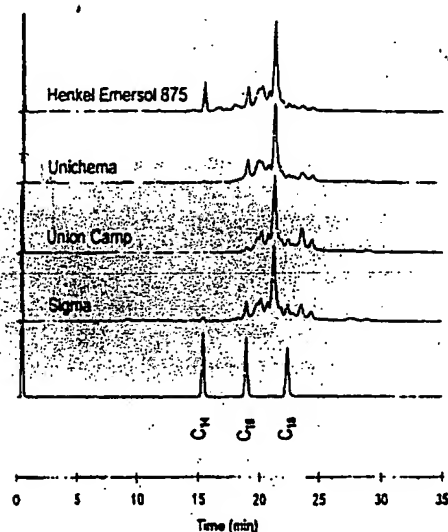


Figure 2. Gas chromatograms of ISA from four vendors.

Table 2
Fatty Acid Composition of ISA and Solubility of Imiquimod in ISA at 25°C

ISA Manufacturer and Lot No.	Solubility (%w/w)	Fatty Acid Profile (Area Percent)		
		< C_{18}	C_{18}	> C_{18}
Henkel				
3AH01	17.0	26	62	11
7BH01	15.8	27	65	10
Union Camp				
94A11FE4	12.1	4	67	30
95U15FE5	13.6	5	65	31
Unichema				
295945	14.7	17	66	17
090646	15.4	16	68	17
090465	14.0	16	66	17
090614	12.6	16	68	16
Sigma				
65H0037	14.3	15	64	22

a greater percentage of fatty acids less than C_{18} . As the fatty acid composition shifts to greater percentages of long-chain fatty acids, imiquimod solubility decreases. This dependency was seen with ISA from Henkel; this vendor supplies ISA with the highest observed percentage of fatty acids less than C_{18} in which the solubility of imiquimod is the greatest. The lowest measured solubility was observed in ISA supplied from Union Camp, with about 5% of the fatty acids less than C_{18} .

Temperature Dependence of Imiquimod Solubility in ISA

The solubility of imiquimod was measured in ISA (Emersol 875 grade) as a function of temperature and the results are plotted in Fig. 3. At temperatures greater than 40°C, the solubility of imiquimod decreased. This decline in solubility was due in part to a changing pK_a with temperature. Other factors may play a role in the solubility of imiquimod in ISA, such as the temperature stability of the complex. The pK_a of imiquimod cannot be determined in water because the intrinsic solubility (0.00060 mg/ml) is too low for accurate titration. However, solvent blends of methanol in water were able to solubilize a sufficient concentration of imiquimod for titration. The apparent pK_a values were extrapolated to zero methanol concentration to determine the pK_a of imiquimod in water, as shown in Fig. 4. The pK_a of imiquimod in water was determined at several temperatures and declined in

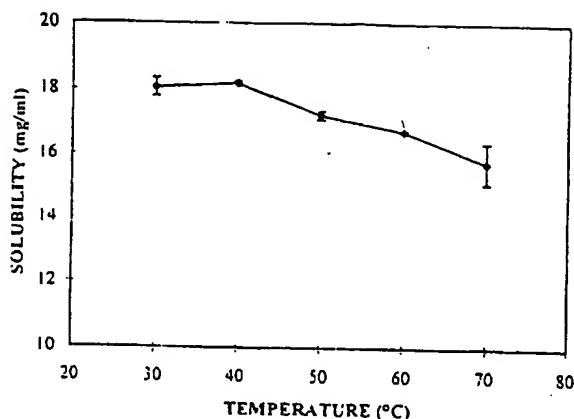


Figure 3. Solubility of imiquimod in ISA as a function of temperature ($n = 3$ and error bars represent standard deviation of the mean).

a similar trend as solubility (Table 3). Imiquimod is thought to complex with fatty acids within ISA through hydrogen bonding and the strength of this hydrogen bond is dependent on the base strength of imiquimod. The dissociation constants of weak bases are affected by temperature, and the pK_a of imiquimod decreases with increasing temperature, resulting in a weaker hydrogen bonded complex with fatty acids. Despite this temperature dependence of the solubility, imiquimod has sufficient solubil-

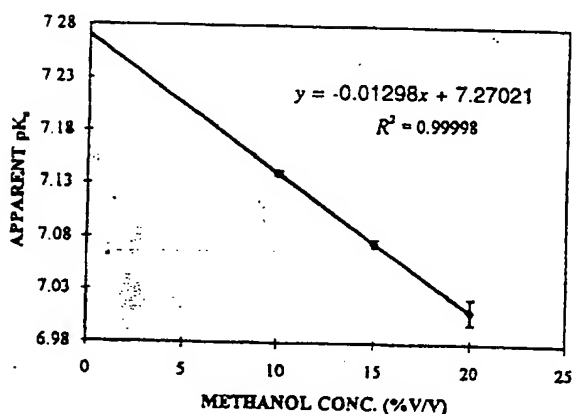


Figure 4. pK_a determination of imiquimod in water at 22°C (room temperature) by extrapolation to zero methanol concentration. The standard deviation is reported as the difference between the apparent pK_a at the lowest methanol concentration and the y-intercept.

Table 3

Comparison of Dissociation Constant of Imiquimod in Water and Solubility of Imiquimod in ISA as a Function of Temperature

Temperature (°C)	pK_a	Solubility in ISA (mg/ml)
Room temperature (~22)	7.27 ± 0.13	nd
25	7.16 ± 0.13	17.0 ± 0.08
30	6.97 ± 0.10	18.05 ± 0.27
40	6.95 ± 0.15	18.17 ± 0.07
50	6.78 ± 0.13	17.21 ± 0.07
60	6.67 ± 0.11	16.69 ± 0.04
70	nd	15.73 ± 0.62

nd: Not determined.

ity in ISA to achieve a 5% topical emulsion at all relevant processing and storage temperatures.

Stabilization of Emulsions Based on ISA and Water

Once ISA was selected as the solubilizer for the emulsion, a suitable surfactant system was sought to stabilize this interface. Combinations rather than single surfactants were evaluated because a more effective and more closely packed interfacial film is expected. The two surfactants can exist side-by-side at the interface with the penetration of one surfactant into the interfacial layer of the other. One surfactant was chosen with a high hydrophile-lipophile balance (HLB) and another with a low HLB to provide a final HLB value of 15–16, which is the calculated HLB value for the main components for the oil phase. Two additional formulations were prepared with surfactant systems at an HLB value of 12 to determine if a lower HLB resulted in a more stable formulation. The surfactant systems studied are shown in Table 4. Although all emulsions with these surfactant systems showed some degree of phase separation after 3 months at 40°C, the emulsion with polysorbate 60 and sorbitan monostearate appeared to be more homogeneous and smoother than emulsions with the other surfactant systems. Therefore, this surfactant system was selected for further stability assessment. The emulsion was further stressed by temperature cycling for 10 cycles between 4 and 40°C, and did not exhibit phase separation. This formulation proved to be stable for at least 24 months at both 4 and 20°C. However, separation was evident at 30°C after about 9 months. The surfactant combination of polysorbate 60 and sorbitan monostearate appeared to

Table 4
Surfactant Systems Evaluated for Imiquimod Cream 5%

Surfactant System Number (HLB)	Ingredient	Concentration (%w/w)
1 (13.4)	Polysorbate 60	3.4
	Sorbitan monostearate	0.6
2 (15.1)	Sorbitan monopalmitate	0.3
	Polysorbate 40	4.7
3 (13.5)	Polyoxyethylene 50 stearate	3.9
	Sorbitan monostearate	1.1
4 (15.7)	Polyoxyethylene 2 stearyl ether	0.25
	Polyoxyethylene 20 stearyl ether	4.75
5 (15.9)	Polyoxyl 40 stearate	4.6
	Glycerol monostearate	0.4
6 (15.9)	Sorbitan monostearate	0.4
	Polyoxyl 40 stearate	4.6
7 (16.0)	Polysorbate 60	2.0
	Polysorbate 20	3.0
8 (15.2)	Polyoxyethylene 100 stearate	2.8
	Glycerol monostearate	1.2
9 (15.5)	Polysorbate 60	2.8
	Polyoxyl 40 stearate	1.2
10 (15.1)	Polysorbate 40	4.8
	Propylene glycol monostearate	0.2
11 (14.7)	Polysorbate 60	3.8
	Polysorbate 65	0.2
12 (12.0)	Polysorbate 60	3.6
	Sorbitan monostearate	1.4
13 (12.0)	Polysorbate 40	3.0
	Sorbitan monopalmitate	2.0

be a better choice than the other surfactants screened on the basis of the physical appearance and stability data.

Initial formulations were not sufficiently viscous to maintain physical stability. Therefore, a series of viscosity-enhancing agents were evaluated including methylcellulose, ethylcellulose, hydroxypropylcellulose, colloidal silicon dioxide, magnesium aluminum silicate, Pluronic F127, xanthan gum, and carbomer 934. Xanthan gum (0.5%) and Pluronic F127 (8.6%) each increased the viscosity and physical stability of the formulation. Xanthan gum was chosen over Pluronic F127 because of a lower effective concentration and less irritation in a topical rabbit irritation study. With the addition of 0.5% xanthan gum in the formulation, the apparent viscosity increased to at least 10,000 cps and separation did not occur after 3 months at 40°C.

Other excipients in the formulation are oil phase modifiers that include white petrolatum, cetyl alcohol, and stearyl alcohol. White petrolatum was used to add consistency to the vehicle and improve the emollient properties of the formulation.

It is a common ingredient in topical formulations because it promotes the retention of the cream in contact with the skin but is not readily absorbed. Cetyl and stearyl alcohols also provide some emollient properties, but primarily improve texture and physical stability of the emulsion. They are both water-absorptive emulsifiers that lubricate the skin.

The continuous phase of this o/w emulsion was predominantly water. Therefore, an acceptable preservative system was required to meet the USP preservative effectiveness test (PET). The best antimicrobial activity in screening experiments was seen with the combination of methylparaben (0.2%) and propylparaben (0.02%) with either sodium benzoate and benzoic acid (0.5% each), benzoic acid (0.5%) and sodium propionate (2%), or benzyl alcohol (2%). Sodium propionate was eliminated because its addition to the formulation prevented a stable emulsion. Benzyl alcohol formulations were efficacious

against both bacteria and fungi with acceptable PET results. The benzoate formulations also passed the USP criteria, but were less effective. The marketed product contains benzyl alcohol and not the benzoates because benzyl alcohol acts as a cosolvent to aid in the dissolution of imiquimod in ISA.

Release Tests on Imiquimod Cream

The purpose of in vitro release testing was to demonstrate that imiquimod was released from the cream formulation. Dilute HCl (0.1 N) was used as the receptor phase to maintain sink conditions. The amount of imiquimod released as a function of time was measured. The data were analyzed according to a modified form of the Higuchi equation (11):

$$Q = (2ADC_0t)^{1/2} \quad (1)$$

where Q is the total amount diffused, A is the total concentration of drug in the matrix, D is the diffusion coefficient of drug in the matrix, C_0 is the saturation solubility of drug in the formulation, and t is time. The total amount of drug released was plotted versus the square root of time. Typical results are presented in Fig. 5. The steady-state flux was calculated from the slope of the line and this was reported as the apparent release constant in $\text{mg}/\text{cm}^2\text{min}^{0.5}$. About 50% of the initial amount of imiquimod was released after 6 hr, and the plot remained linear.

These experiments were not meant to correlate with transport across human skin, but rather to demonstrate that the release rate was not influenced by processing or

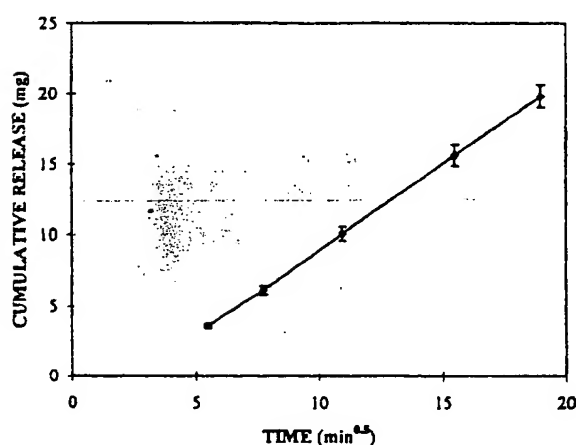


Figure 5. Release of imiquimod from the cream as a function of time as measured with a Franz cell ($n = 6$ and error bars represent standard deviation of the mean).

storage conditions (12,13). The mechanism of imiquimod release from the formulation between an in vitro and in vivo experiment into the skin may be different. For the in vitro situation for which drug release is measured with a Franz cell, the release medium diffuses across a porous membrane into the formulation matrix. The rate-limiting step in these experiments is diffusion from the formulation matrix. However, the formulation is spread as a thin layer on the skin to enhance the surface area exposure and accelerate the release from the formulation. The rate-limiting step is transport across the skin rather than diffusion from the formulation matrix. The target site for imiquimod is the Langerhans cells within the skin, and by preventing systemic absorption, systemic side effects can be minimized. Therefore, the formulation must release and deliver imiquimod into the skin.

Pharmacological Effectiveness of Imiquimod Topical Cream

Once a physically stable emulsion of imiquimod was developed which gave acceptable release characteristics in vitro, it was tested in animal models to prove that it delivered the drug to the skin in vivo. A 5% ISA emulsion of imiquimod was applied to the flanks of male hairless mice and skin biopsies were taken at various times after dosing. The biopsied tissues were analyzed for the levels of three cytokines that are induced by imiquimod (IFN- α , IL-6, and TNF- α), and the results were compared to control tissues taken from sites not exposed to drug.

Figure 6 plots the results of this pharmacologic testing, using $n = 3$ and error bars representing the standard error of the mean. The first set (control) shows the baseline levels of these cytokines prior to drug exposure. In each case a statistically significant increase in the level of cytokines was found after application of the topical imiquimod emulsion. Because this is not a direct measure of the drug concentration locally, but an indirect measure of its subsequent activity, the time to peak concentration varies for each cytokine, and the activity can extend well beyond that expected from the drug's pharmacokinetic half-life.

The ISA provides an excellent vehicle for topical delivery of imiquimod, but may also act as a penetration enhancer. Fatty acids are known to enhance the penetration of drug molecules through the skin by forming complexes with basic drugs (14). An increase in the amount of solubilizer, which reduces the thermodynamic activity of the solute (drug), typically reduces the flux into the skin (5). Preliminary experiments in our laboratory indi-

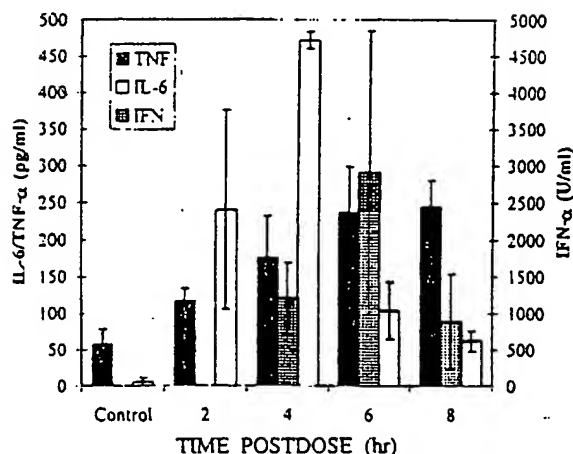


Figure 6. Induction of cytokines IL-6 (white), TNF- α (dark), and IFN- α (shaded) after topical dosing on mice as a function of time. Each bar represents the standard error of the mean for $n = 3$.

cate that the percent imiquimod delivered through hairless mouse skin is increased when the ratio of ISA/drug is increased. This would be consistent if the solubilizer was acting as a penetration enhancer in addition to a solubilizer/vehicle.

CONCLUSION

These results show that once an effective solvent was found to prepare an emulsion of imiquimod in the fully dissolved state, a topically active formulation could be made for this drug. ISA has been shown to be a useful water-immiscible excipient that is more stable than the more commonly used unsaturated fatty acids (oleic acid, linoleic acid). The resulting cream can be stabilized by typical emulsifying surfactants and has excellent physical stability to droplet coalescence with the addition of a viscosity-enhancing polymer. For basic drugs that are not soluble in water or common emulsion oils, fatty acids may represent a useful topical formulation aid.

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